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**SPOTLIGHT REVIEW**

# Inflammation and metabolic cardiomyopathy

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**Abstract** Excessive feeding is associated with an increase in the incidence of chronic metabolic diseases, such as obesity, insulin resistance, and type 2 diabetes. Metabolic disturbance induces chronic low-grade inflammation in metabolically-important organs, such as the liver and adipose tissue. Many of the inflammatory signalling pathways are directly triggered by nutrients. The pro-inflammatory mediators in adipocytes and macrophages infiltrating adipose tissue promote both local and systemic pro-inflammatory status. Metabolic cardiomyopathy is a chronic metabolic disease characterized by structural and functional alterations and interstitial fibrosis without coronary artery disease or hypertension. In the early stage of metabolic cardiomyopathy, metabolic disturbance is not accompanied by substantial changes in myocardial structure and cardiac function. However, metabolic disturbance induces subcellular low-grade inflammation in the heart, and in turn, subcellular component abnormalities, such as oxidative stress, mitochondrial dysfunction, endoplasmic reticulum stress, and impaired calcium handling, leading to impaired myocardial relaxation. In the advanced stage, the vicious cycle of subcellular component abnormalities, inflammatory cell infiltration, and neurohumoral activation induces cardiomyocyte injury and death, and cardiac fibrosis, resulting in impairment of both diastolic and systolic functions. This review discusses some recent advances in understanding involvement of inflammation in metabolic cardiomyopathy.

**Keywords** Inflammation • Metabolic cardiomyopathy • Innate immune system • Autophagy

**This article is part of the Spotlight Issue on Metabolic Cardiomyopathies.**

## 1. Introduction

Excessive feeding is associated with an increase in the incidence of chronic metabolic diseases, such as obesity, insulin resistance and type 2 diabetes.[1] Insulin resistance refers to the decrease in insulin action on target tissues, such as liver, adipocytes, and skeletal muscle. Insulin resistance is a risk factor of left ventricular dysfunction and heart failure, and is a hallmark of type 2 diabetes. Metabolic cardiomyopathy develops in the context of a wide spectrum of pathological conditions and is associated with systemic metabolic disorders acquired during adulthood.[2] Metabolic cardiomyopathy, in particular, is characterized by structural and functional alterations and interstitial fibrosis without coronary artery disease or hypertension.[3] Metabolic disturbance triggered by nutrients, such as hyperglycaemia, and increased free fatty acid levels, induces chronic low-grade inflammation in metabolically-important organs, such as the liver and adipose tissue.[1] Pro-inflammatory cytokines are released and immune cells are infiltrated in adipose tissue. The immune cells infiltrating adipose tissue contribute to the production and secretion of the pro-inflammatory mediators and promote both the local and systemic pro-inflammatory status.[4] The pro-inflammatory mediators can exacerbate systemic insulin resistance and contribute to cardiac insulin resistance.[5] Metabolic disturbance induces subcellular low-grade inflammation in the heart. The early stage of metabolic cardiomyopathy is not accompanied by substantial changes in myocardial structure and cardiac function. However, in turn, the systemic metabolic disorders induce subcellular component abnormalities, such as oxidative stress, mitochondrial dysfunction, endoplasmic reticulum (ER) stress, and impaired calcium handling, leading to impaired myocardial relaxation. In the advanced stage, increased subcellular component abnormalities, inflammatory cell infiltration, neurohumoral activation, and their vicious cycle, induce cardiomyocyte injury and death and cardiac fibrosis, resulting in impairment of both diastolic and systolic functions (*Figure 1*).[6,7]

Inflammatory signalling in cardiomyocytes usually occurs as an early response to myocardial injury and entails mitochondrial reactive oxygen species (ROS) overproduction.[3] The innate immune system plays a crucial role in acute inflammation caused by microbial infection or tissue damage.[8] The innate immune system is also involved in chronic metabolic inflammation.[9] Pattern recognition receptors (PRRs), including the Toll-like receptors (TLRs), are responsible for detecting pathogens

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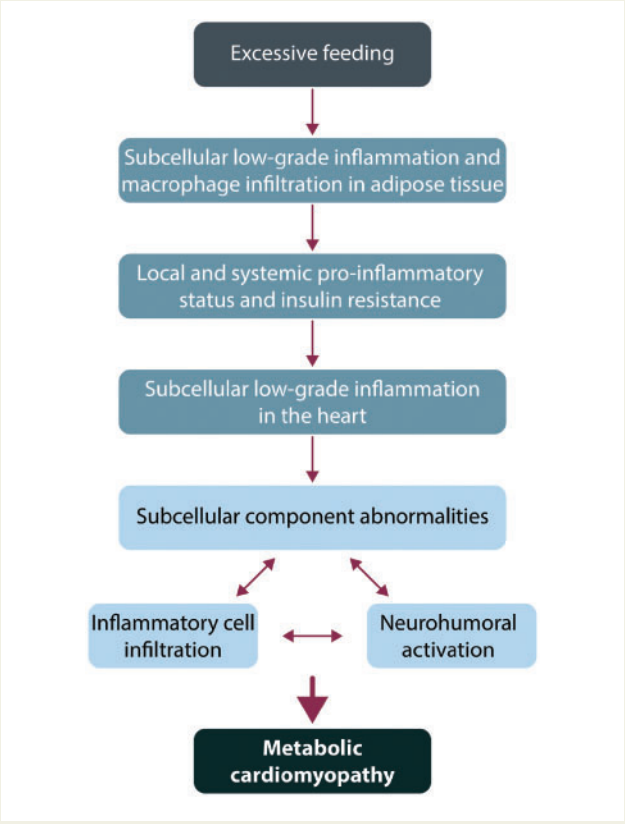


**Figure 1** The development and progression of metabolic cardiomyopathy. Inflammation and insulin resistance in adipose tissue by metabolic disturbance induce systemic inflammation and insulin resistance, resulting in subcellular low-grade inflammation, and in turn, subcellular component abnormalities in the heart. The vicious cycle of increased subcellular component abnormalities, inflammatory cell infiltration, neurohumoral activation induce the adverse cardiac remodelling in metabolic cardiomyopathy.

through recognition of conserved molecular motifs, called pathogen-associated molecular patterns (PAMPs). PRRs also recognize endogenous molecules released from damaged cells, termed damage-associated molecular patterns (DAMPs). TLRs are classically divided into two groups: cell surface TLRs, such as TLR2 and TLR4, and endosomal TLRs, such as TLR9. TLR2, TLR4, and TLR9 recognize lipoproteins, lipopolysaccharide, and DNA, respectively.[8] TLR2 on hematopoietic cells derived from bone marrow mediates myocardial ischaemia/reperfusion injury, such as myocardial infarction[10] and endothelial dysfunction of coronary arteries[11]. TLR2 ablation[10,11] or TLR2 inhibition by an anti-TLR2 antibody[10,12] reduces infarct size in *in vivo* ischaemia/reperfusion model. Ablation or inhibition of TLR2 also attenuates left ventricular remodelling in cardiomyopathy induced by transverse aortic constriction (TAC), angiotensin, and doxorubicin.[13–15] TLR4-deficient mice also show reduced infarct size, attenuated adverse remodeling and decreased inflammation.[16,17] TLR9-deficient mice subjected to TAC display attenuation of inflammatory cell infiltration and heart failure compared with wild-type mice subjected to TAC.[18] These findings suggest that TLRs play an important role in development of cardiac dysfunction. The nucleotide-binding and oligomerization domain-like receptor family, pyrin domain containing protein 3 (NLRP3) inflammasome is the most fully characterized of inflammasomes and contains the adaptor protein, ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain); the pro-inflammatory caspase, caspase-1; and NLRP3. Once assembled, inactive pro-caspase-1 in the inflammasome complex is auto-processed by proximity to active caspase-1, which subsequently induces the maturation of pro-interleukin (IL)-1β or pro-IL-18 to their active forms.[19,20] Caspase-1 is upregulated in murine and human failing hearts.[21] Genetic ablation of Nlrp3 in a model of dilated cardiomyopathy reduces pro-inflammatory cytokine maturation and cardiac inflammation, and improves systolic performance.[22] These findings suggest that NLRP3 inflammasome also plays an important role in development of cardiac dysfunction.

In this manuscript, we discuss some recent advances in the understanding of involvement of inflammation in metabolic cardiomyopathy.

# 2. Metabolism

## 2.1 Metabolic regulation in normal hearts and failing hearts

In the normal hearts, >95% of ATP generated in the heart is derived from oxidative phosphorylation in the mitochondria. Free fatty acids, predominantly long-chain fatty acids, are preferred as energy substrates in the adult heart. Long-chain fatty acids enter in the cytosol by passive diffusion or fatty acid translocase (FAT)/cluster of differentiation (CD)36.[2] Approximately 70 to 90% of cardiac ATP is produced by the oxidation of fatty acids. The remaining 10 to 30% comes from the oxidation of glucose and lactate, as well as small amounts of ketone bodies and certain amino acids.[23] Glucose uptake is mostly regulated by the glucose transporter (GLUT)1 and GLUT4, of which GLUT4 is the most abundant, in cardiomyocytes. Glucose utilization is reduced by increased fatty acid oxidization via Randle cycle.[24] Insulin is the main anabolic hormone in mammals and is essential for metabolic homeostasis.[1] The binding of insulin to insulin receptor on the cell surface activates the phosphorylation of tyrosine residues at the insulin receptor substrate 1 (IRS-1), which activates the phosphatidylinositol 3-kinase (PI3K)-Akt pathway, and then induces GLUT4 translocation to the cell membrane (*Figure 2*). Insulin signalling also promotes signal transduction via mitogen-activated protein kinase, which contributes to growth and remodelling.[7] The PI3K is also capable of promoting FAT/CD36 translocation to the cell membrane. The normal heart exhibits remarkable fuel flexibility, switching between fatty acids and glucose according to human body's nutritional state and physical activity. After a high carbohydrate supply, cardiac energy substrate shifts towards glucose usage by the increased insulin levels.[2] AMP-activated protein kinase (AMPK) is an energy-sensing enzyme that is activated when cellular energy levels are low. AMPK activation improves insulin sensitivity and glucose homeostasis.[25] AMPK promotes myocardial GLUT4 expression and translocation to the plasma membrane in a similar way to insulin.[26]

Impaired oxidative phosphorylation can reduce cardiac function by providing an insufficient supply of ATP to cardiomyocytes.[27] In the failing hearts, fatty acid uptake and fatty acid transporters are reduced in the animal heart failure models.[23] In humans, the higher NYHA classes are associated with a more severe limitation in oxidative phosphorylation capacity.[28] The concentrations of phosphocreatine and ATP are reduced in failing myocardium with dilated cardiomyopathy and correlates with left ventricular volumes, ejection fraction, and clinical status.[29]

## 2.2 Insulin resistance

Insulin resistance means the inability of insulin to promote its metabolic actions in organs, such as adipose tissue, skeletal muscle and liver. Insulin

resistance is a hallmark of obesity and type 2 diabetes. Insulin-resistant states exhibit impaired insulin-mediated glucose uptake in muscle and adipocytes, impaired suppression of hepatic glucose production and impaired suppression of lipolysis.[30] Several mechanisms, including increased lipolysis, higher free fatty acid release, reduced glucose uptake, decreased secretion of adiponectin, an anti-inflammatory adipokine, increased secretion of leptin, and the increased secretion of pro-inflammatory cytokines, may cause impaired insulin sensitivity. Tumour necrosis factor (TNF)-α, IL-6 and free fatty acids activate intracellular kinases that induce serine phosphorylation of IRS-1, thereby attenuating insulin signalling and inducing insulin resistance.[4] Free fatty acids stimulate adipose tissue inflammation through TLR4 pathways, resulting in insulin resistance. Fetuin-A, a liver secretory glycoprotein, acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance and stimulates the production of inflammatory cytokines from adipocytes and macrophages.[31] Free fatty acids-fetuin-A complex activates TLR4 signalling pathway, resulting in NF-κB and c-Jun N-terminal kinase (JNK) activation.[32] JNK activity is abnormally elevated in obesity. An absence of JNK1 results in decreased adiposity, significantly improves insulin sensitivity and enhances insulin receptor signalling capacity.[33] suppressor of cytokine signalling 3 (SOCS3), protein tyrosine phosphatase-B1 (PTP-1B), and JNK inhibit insulin signalling (*Figure 2*).[5,32] Increased ER stress, ROS generation, and mitochondrial dysfunction have been suggested to contribute to the development of insulin resistance.[30] Importantly, immune cells infiltrating adipose tissue contribute extensively to the production and secretion of the pro-inflammatory mediators and promote both the local and systemic pro-inflammatory status.[4] The pro-inflammatory mediators can exacerbate systemic insulin resistance and contribute to cardiac insulin resistance.[5]

## 2.3 Metabolic regulation in metabolic cardiomyopathy

Fatty acid uptake and oxidation are increased in obesity and insulin resistance.[34–36] In states of insulin resistance and/or type 2 diabetes, FAT/CD36 becomes localized to the sarcolemmal membrane, whereas GLUT4 is internalized and returns to its intracellular location, resulting in metabolic inflexibility. Excess lipid accumulation in the heart produces a form of 'lipotoxicity', which refers to toxicity arising from the cellular accumulation of lipid and lipid intermediates, leading to morphological structural alteration, as well as impaired myocardial performance.[37] Lipotoxicity can promote cardiomyocyte apoptosis via increased ROS production and ER stress, leading to the development of heart failure. Global insulin resistance leads to chronic systemic hyperglycaemia, which also contributes to cardiac injury through multiple mechanisms, including overproduction of ROS.[38] Since adiponectin prevents insulin resistance[4] and adiponectin treatment decreases hydrogen peroxide-induced cardiomyocyte hypertrophy,[39] decreased adiponectin secretion from adipose tissue may be involved in development of metabolic cardiomyopathy mediated through impaired insulin signalling in the heart.

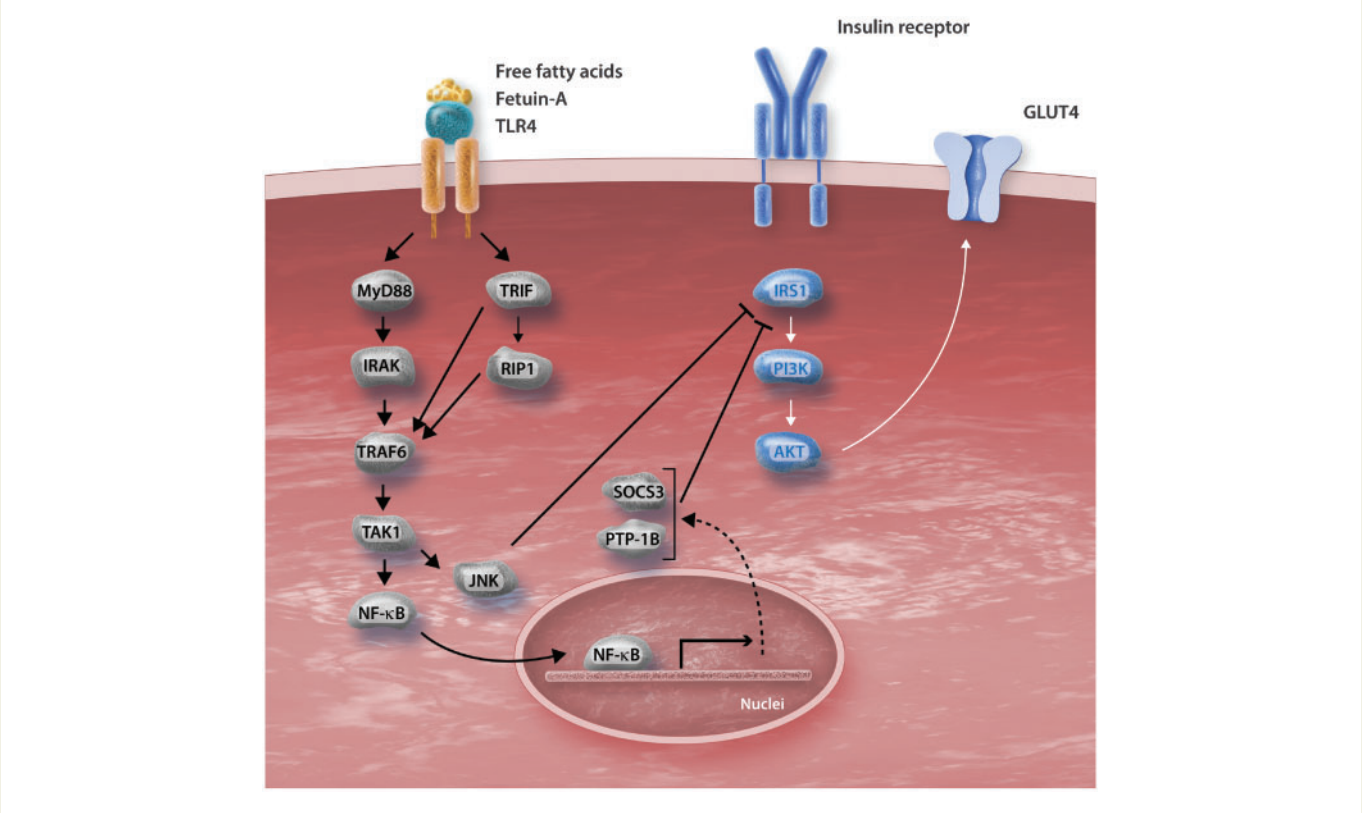


**Figure 2** The mechanism how free fatty acids affect insulin resistance mediated through TLR4 signalling pathway. Fetuin-A is induced by stimulation of free fatty acids through NF-κB pathway and acts as a carrier protein of free fatty acids in the circulation. Free fatty acids-fetuin-A complex activates TLR4 signalling pathway, resulting in insulin resistance.[31] IRAK, IL-1 receptor-associated kinase; RIP1, receptor-interacting protein 1; TAK1, TGF-β activated kinase 1; TRAF6, TNF receptor associated factor 6; TRIF, Toll/IL-1 receptor domain-containing adaptor-inducing interferon-β.

Peroxisome proliferator activator receptor (PPAR)-α is expressed in abundant levels in the heart. Enhanced PPAR-α signalling is especially associated with increased fatty acid oxidation in cardiomyocytes. PPAR-γ co-activator 1α upregulates and improves mitochondrial biogenesis. However, during the advanced stage of metabolic cardiomyopathy, these signal transduction molecules involved in regulating oxidation of fatty acids are decreased.[5,7]

# 3. Involvement of inflammation in metabolic cardiomyopathy

## 3.1 Chronic inflammation in adipose tissue, liver and pancreatic islet

Obesity is associated with a state of chronic low-grade inflammation in metabolically-important organs, such as the liver and adipose tissue. Many of the inflammatory signalling pathways are directly triggered by nutrients, such as circulating lipids.[1] Adipose tissue is both responsive to and responsible for a wide variety of hormonal, inflammatory, and metabolic interactions with other organ. The main function of adipocytes is to store energy in the form of the triglycerides.[38] Adipocyte hypertrophy is an important and early event in the aetiology of adipose tissue inflammation. With adipocyte hypertrophy, adipocytes increase the production of pro-inflammatory mediators, such as leptin, IL-6 and monocyte chemoattractant protein 1 (MCP-1), and decrease anti-inflammatory or insulin sensitizing factors, such as IL-10 and adiponectin.[4] Macrophages are accumulated in the adipose tissue of obese mice and humans.[40,41] Obesity induces a phenotype switch in adipose tissue macrophage polarization from M2 (anti-inflammatory and tissue repairing) state to M1 (inflammatory) state that contributes to adipose tissue inflammation and insulin resistance. Pancreatic islets of type 2 diabetic patients and rodent models exhibit cell infiltration. IL-1β is released from macrophages. IL-1β contributes to pancreatic islet β-cell dysfunction in type 2 diabetes. IL-1β deficiency or IL-1 receptor antagonist improves β-cell function.[19]

## 3.2 Inflammation in metabolic cardiomyopathy

Inflammation has been recognized as a key pathogenic feature of lipid excess and diabetes. The nuclear factor-κB (NF-κB) is a primary regulator of inflammatory responses. NF-κB induces the expression of pro-inflammatory cytokines, such as TNF-α, IL-6, pro IL1-β, and pro-IL-18 in the heart. NF-κB can also induce the expression of NLRP3 (*Figure 3*).[3] Exposure of fatty acids or high glucose induces NF-κB activation in cardiomyoctes.[42,43] High fat feeding is also associated with cardiac hypertrophy, inflammation, including upregulation of IL-6, TNF-α, MCP-1, and NF-κB p65 mRNA levels, mitochondrial-dependent ROS production, and cardiac advanced glycation end product (AGE) accumulation in wild-type mouse hearts. These phenotypes are attenuated in receptor for advanced glycation end products (RAGE)-deficient mouse hearts.[44] High fat feeding-induced inflammation and defects in glucose metabolism are attenuated in IL-6-deficient mice.[45] Intra-myocardial inflammation, such as infiltration of TNF-α- and IL-1β-expressing immune cells, is increased in streptozotocin (STZ)-induced type 1 diabetic rat model.[46] TNF-α antagonism attenuates the development of STZ-induced diabetic

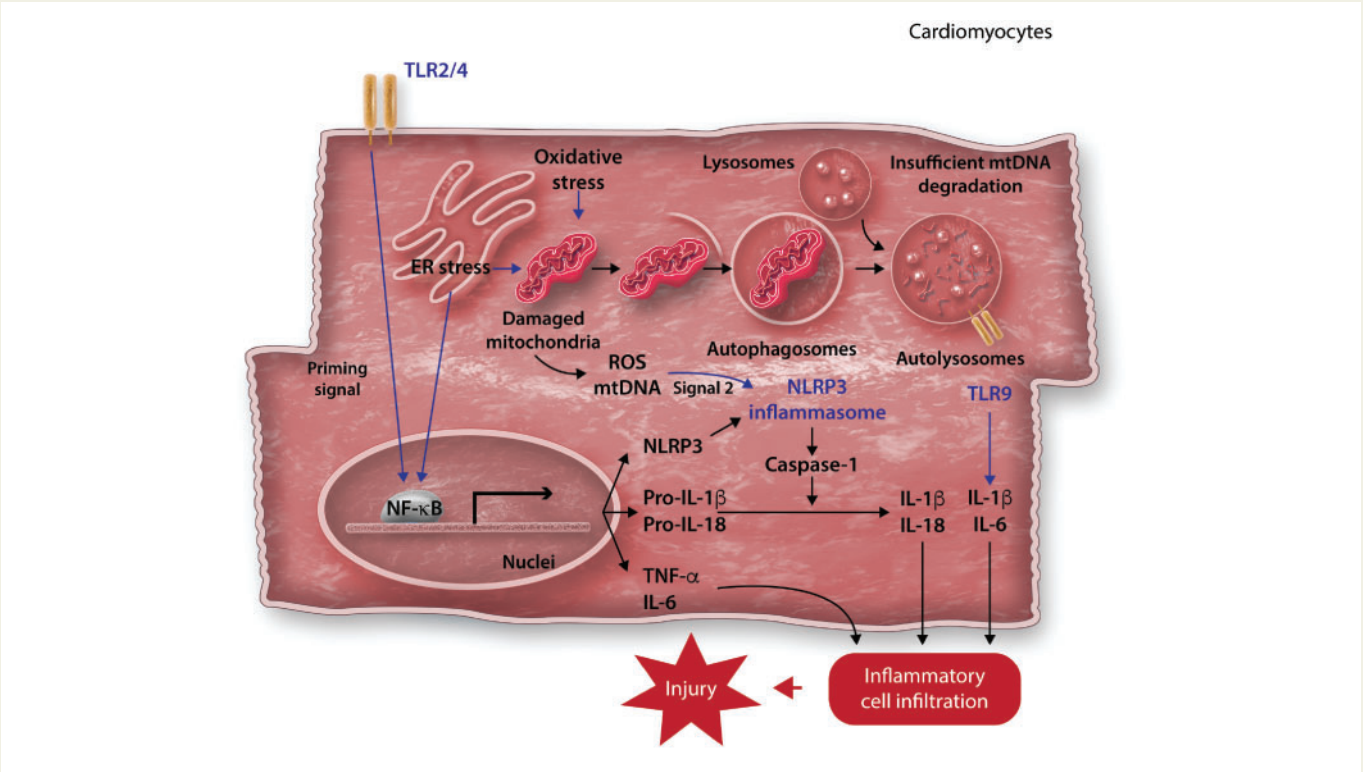


**Figure 3** The mechanisms that lead to inflammation in metabolic cardiomyopathy. It is suggested that the activation of NF-κB signalling mediated through TLR2 and TLR4, TLR9 signalling and NLRP3 inflammasome plays an important role in cardiac inflammation and pathogenesis of metabolic cardiomyopathy. Their activation induces subcellular inflammation and then inflammatory cell infiltration, resulting in cardiac injury.

cardiomyopathy associated with a reduction of intra-myocardial inflammation and cardiac fibrosis.[47] Knockdown of NLRP3 improves cardiac function and reduces mature IL-1β expression in diabetic rat models with a combination of high fat diet and STZ injection.[48] Oxytocin treatment prevents cardiac dysfunction and inhibits apoptosis, fibrosis, ROS production and inflammation in type 2 diabetic *db/db* mouse hearts.[49] However, it is unclear whether myocardial inflammation is involved in cardiac dysfunction in the models of type 2 diabetes. The M1 macrophages secrete inflammatory cytokines that cause reduced systemic and cardiac insulin signalling. The expression of the M1 macrophage marker is associated with development of metabolic cardiomyopathy induced by a Western diet in mice.[50] These findings suggest that myocardial inflammation is involved in the development of metabolic cardiomyopathy. Furthermore, inflammatory cytokines, such as IL-6 and MCP-1, are upregulated in the myocardium before the onset of cardiac dysfunction, and this is accompanied by macrophage infiltration in the lipotoxic cardiomyopathy mouse model. Early non-selective macrophage depletion with clodronate liposomes reduces cardiac inflammatory response and improves cardiac function, suggesting that the early induction of subcellular low-grade inflammation and recruitment of macrophage contribute to the adverse cardiac remodelling.[6] In contrast, bone morphogenetic protein 7, a mediator of monocyte polarization, activates infiltrated monocytes into anti-inflammatory M2 macrophages, thereby attenuating adverse cardiac remodelling in pre-diabetic cardiomyopathy,[51] suggesting that further investigation is required for elucidating the inflammatory responses in the early stage of metabolic cardiomyopathy.

### 3.3 Inflammation in chronic failing hearts

Activation of neurohumoral and sympathetic systems, oxidative stress, mitochondrial dysfunction, ER stress, and impaired calcium handling are involved in development and progression of chronic heart failure.[7] These mechanisms are also activated in the advanced stage of metabolic cardiomyopathy. The link between heart failure and inflammation was first reported in 1990, when circulating levels of TNF were shown to be elevated in severe chronic hearts.[52] In most patients with chronic heart failure, circulating cytokines are elevated and the elevated cytokine levels correlate with the severity of heart failure and prognosis.[53,54] Several animal models suggest that pro-inflammatory mediators play an important role in the development and progression of heart failure.[55] However, many studies of anti-inflammatory therapy for heart failure have shown neutral or negative effects on outcomes of patients with heart failure.[56]

We observed massive cell infiltration of leucocytes including macrophages and neutrophils in wild-type mouse failing hearts with systolic dysfunction in response to pressure overload.[18] Both TLR2 and TLR4 are strongly upregulated in chronic dilated cardiomyopathy and heart failure. Activation of TLR2 and TLR4 eventually leads to reduction of ejection fraction through NF-κB-dependent mechanisms.[3,57] TLR9-deficient mice subjected to pressure overload by TAC display attenuation of inflammatory cell infiltration and heart failure compared with wild-type mice subjected to TAC.[18] NLRP3 inflammasome effector, caspase-1 is upregulated in murine and human failing hearts.[21] Genetic ablation of Nlrp3 in the cardiac-specific calcineurin transgenic mice, which exhibit dilated cardiomyopathy, reduces pro-inflammatory cytokine maturation and cardiac inflammation, as well as improving systolic performance.[22] These findings suggest that inflammation mediated by innate immune system is involved in the pathogenesis of heart failure. In contrast, tissue stress or malfunction induces an adaptive response, which is referred to as parainflammation.[58] In the early stage of chronic heart failure, the primary purpose of the inflammatory response in the heart is to resolve tissue injury, thereby allowing the heart to adapt to the abnormal conditions, and ultimately to restore homeostasis and cardiovascular function.[59] The mechanisms of inflammation and subsequent initiation of tissue repair have been relatively well defined in the acute setting after cardiac injury.[60] M2 macrophages, but not M1 macrophages, accumulate early time points after TAC in mouse hearts.[61] Further investigation is required to clarify the reasons for neutral or negative results targeting inflammation in chronic heart failure.

## 4. Mechanisms of inflammation in metabolic cardiomyopathy

### 4.1 Activation of inflammation by nutrients in adipocytes and macrophages

Increased exposure to fatty acids has been proposed as a key activator of both altered metabolic and immune signalling in obesity.[62] Saturated fatty acids, but not unsaturated fatty acids, induce NF-κB activation and expression of inflammatory markers mediated through TLR4 in monocyte/macrophage cells.[63] Activation of TLR4 with free fatty acids stimulates NF-κB signalling and expression of inflammatory cytokine genes, such as TNF-α and IL-6, and provokes insulin resistance in adipocytes.[64] Free fatty acids can cause activation of macrophages via the TLR2/4-JNK signalling cascade as well.[65] These findings indicate that TLRs are involved in cytokine activation via NF-κB and JNK in adipose cells and macrophages. Furthermore, obesity-related adipocyte degeneration causes the release of cell-free DNA, which promotes adipose tissue macrophage accumulation via TLR9.[66] These findings suggest that free fatty acids promote inflammation mediated through TLRs in adipose cells and macrophages.

A high fat diet reduces AMPK activity, leads to attenuated autophagy and mitochondrial ROS generation and then activates the NLRP3 inflammasome in macrophages. Fatty acid-induced NLRP3 inflammasome activation interferes with insulin signalling.[67] Induction of high fat diet-induced obesity causes marked caspase-1 activation in adipose tissue and liver, and the caspase-1 activation is reduced in NLRP3-deficient mice.[20] These findings suggest that the NLRP3 inflammasome contributes to obesity-induced inflammation and insulin resistance. It remains unclear what is the endogenous inducer of NLRP3 inflammasome activation in obesity, but an enhanced generation of saturated fatty acids and ceramides leads to the activation of the NLRP3 inflammasomes, resulting in insulin resistance and lipotoxicity of metabolic syndrome.[20,67] Oxidized mitochondrial DNA (mtDNA) into the cytosol during mitochondrial dysfunction may be involved in NLRP3 inflammasome activation.[68,69] Adipocytes also secrete leucotrienes by a high fat diet. Leucotrienes produced by adipocytes play an important role in attracting macrophages and T cells.[70]

### 4.2 Activation of inflammation by nutrients in the hearts

It is suggested that systemic pro-inflammatory status and insulin resistance induced by inflammation in adipocytes and macrophages promote cardiac low-grade inflammation.[2,3,7] High fat diet-induced obese mice exhibit the high protein expression levels of TLR4 and myeloid differentiation primary response gene 88 (MyD88) and macrophage infiltration in the heart.[45] TLR4-deficient mouse hearts show lower triglyceride accumulation during the early stages of diabetes, as well as restricted leucocyte infiltration.[71] Gene silencing of TLR4 in STZ-injected mice

prevents hyperglycaemia-induced myocardial apoptosis and the suppression of hyperglycaemia-induced apoptosis by TLR4 is associated with attenuation of oxidative stress to the cardiomyocytes.[72] TLR2 participates in the mechanism of ROS-induced activation of NF-κB in cardiomyocytes.[73] However, it is required to examine whether TLRs activation by nutrients is involved in cardiac dysfunction in metabolic cardiomyopathy. NLRP3 gene silencing ameliorates cardiac remodelling and dysfunction in diabetic rats,[48] suggesting that NLRP3 inflammasome is also involved in cardiac inflammation and cardiac dysfunction in metabolic cardiomyopathy. The cyclic GMP-AMP synthase is a cytosolic DNA sensor and activates the adaptor STING, leading to activation of innate immune responses.[74] However, there are no reports that cyclic GMP-AMP synthase-STING pathway is involved in inflammation in metabolic cardiomyopathy.

## 4.3 Other mechanisms that promote metabolic cardiomyopathy

### 4.3.1 Oxidative stress and mitochondrial dysfunction

Mitochondria are crucial organelles for cellular homeostasis and survival, participating in energy production and intracellular transfer, as well as in the regulation of redox and calcium homeostasis, free fatty acid oxidation and apoptosis.[75] Because of the high energy demand in the heart, mitochondria comprise at least 30% of the cardiomyocyte volume.[76] Both hyperglycaemia and hyperlipidaemia can hyperpolarize mitochondria causing electron slippage and enhanced ROS production.[77,78] Increased mitochondrial ROS production and damaged mitochondrial structure and function are commonly seen in the hearts of both type 1 and type 2 diabetic patients and animal models.[79] Various antioxidants or anti-inflammatory compounds reduce diabetic heart injury in animal studies, indicating that ROS play an important role in pathogenesis of diabetic cardiomyopathy.[79–84] Peroxynitrate also contributes to cytokine-induced myocardial contractile failure and triggers cardiomyocyte apoptosis.[85,86] Mitochondria in the failing myocardium exhibit misalignment, disorganized cristae, reduced mitochondrial density, membrane disruption and aggregation. The size and number of mitochondria are decreased and increased in the failing myocardium compared with normal myocardium, respectively.[87,88] ROS production from dysfunctional mitochondria intensifies oxidative damage, such as damage of macromolecules, namely proteins, lipids and DNA, in a vicious amplifying cycle of mitochondrial dysfunction and ROS production, leading to cell death.[78,89] These findings suggest that oxidative stress and mitochondrial dysfunction are involved in the advanced stage of metabolic cardiomyopathy.

### 4.3.2 ER stress

The ER has an important role in protein processing and lipid metabolism. Accumulation of unfolded proteins in the ER, known as ER stress, can trigger an adaptive response, known as the unfolded protein response (UPR). The UPR increases the ability of the ER to fold and translocate proteins. The UPR consists of three main branches controlled by the ER membrane proteins protein kinase-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6. UPR induces autophagy, which is important for degradation of misfolded proteins.[62] ER stress is linked with chronic metabolic diseases, including obesity, insulin resistance, and diabetes.[90] ER stress induces inflammation by activating JNK and/or NF-κB signalling, such as IRE1-mediated activation of JNK, IRE1, and PERK-mediated NF-κB signalling pathway.[1,62] Excessive or prolonged ER stress can lead to cell death mediated by apoptosis.[91] Since apoptotic cell death is a trigger for the recruitment of macrophages and other inflammatory cells, ER stress-induced apoptosis may also play a role in increased inflammatory signalling.[92]

### 4.3.3 Impaired calcium handling

Alteration in calcium handling is the hallmark for the contractile dysfunction seen in heart failure. Decreased contractility in metabolic cardiomyopathy is associated with calcium homeostasis in the cardiomyocytes. Sarcoplasmic reticulum plays a key role in excitation-contraction coupling in the heart. During relaxation, $Ca^{2+}$ is transported into the sarcoplasmic reticulum by the SERCA2a (sarco/ER $Ca^{2+}$ ATPase 2a). Excitation-contraction coupling is altered in diabetic cardiomyocytes. The SERCA2a activity is decreased in the failing diabetic cardiomyocytes.[93,94] Calcium sparks in *db/db* cardiomyocytes are less frequent than in control littermate cardiomyocytes, partly because of the reduced sarcoplasmic reticulum calcium load but also because of the reduced expression of $Ca^{2+}$ release channel.[95] The $Ca^{2+}$ release channel becomes leaky during diabetes and this defect may be responsible for the reduced sarcoplasmic reticulum calcium load in STZ-induced diabetic rats.[96] These findings suggest that impaired calcium handling is involved in development and progression of metabolic cardiomyopathy.

### 4.3.4 Renin angiotensin aldosterone system activation

Both angiotensin II and aldosterone induce serine phosphorylation of IRS-1 and activate the mechanistic or mammalian target of rapamycin (mTOR) complex 1 (mTORC1)/S6 kinase 1 pathway, leading to systemic, and cardiac insulin resistance. Increased activation of renin angiotensin aldosterone system in states of insulin resistance or hyperinsulinaemia has an important role in the development of metabolic cardiomyopathy.[7,97] Both angiotensin II and aldosterone cause significant cytosolic oxidative stress via trans-activation of nicotinamide adenine dinucleotide phosphate reduced form (NADPH) oxidase and production of ROS. The oxidative stress causes cardiomyocyte cell damage, such as calcium overloading, apoptosis, and fibrosis. Angiotensin II stimulates increased cardiac fibroblast proliferation. Aldosterone signalling via mineralocorticoid receptor promotes cardiac stiffness and impairs cardiac relaxation.[5,7] Angiotensin type 1 receptor antagonist, irbesartan, attenuates cardiac failure by decreasing cardiac inflammation and normalizing metalloproteinase activity in STZ-induced diabetic cardiomyopathy.[98] Neurohumoral activation induces transforming growth factor-β (TGF-β), leading to cardiac fibrosis.[7,99] Activation of TGF-β signalling pathway may be responsible for effects of diastolic dysfunction.

## 4.4 Role of autophagy in metabolic cardiomyopathy

Macroautophagy (commonly referred to as autophagy) is a conserved process from yeast to mammals for the bulk degradation and recycling of long-lived proteins and organelles. Intracellular components are surrounded by double membrane-bound autophagic vesicles which then fuse with lysosomes to form autolysosomes for degradation. Controlled by autophagy-related proteins (Atgs), such as Atg5, Beclin 1, Atg7, and LC3 (microtubule-associated protein 1 light chain 3), autophagy plays an essential role in maintaining cellular homeostasis. Autophagy was initially believed to be a non-selective process. However, it has been revealed that there are selective types of autophagy, including mitochondria-specific autophagy, called mitophagy. The specific substrate for autophagic degradation, p62/SQSTM1, binds to ubiquitinated proteins on the mitochondria via its ubiquitin-associated domain, and binds to LC3 on the

isolation membrane with its WXXL-like motif, resulting in tethering mitochondria to the isolation membrane via the complex selectively.[100] The initiation of autophagy requires the Unc-51-like kinase (ULK) complex. mTORC1 suppresses the ULK complex under nutrient-rich conditions. By starvation or AMPK activation, the ULK complex is activated and translocates to ER.[89] We previously reported that autophagy plays an essential role in maintaining cellular homeostasis in the heart, and a protective role in clearance of damaged cellular components, such as harmful proteins and damaged organelles including damaged mitochondria under the stressed condition using cardiac-specific Atg5-deficient mice.[88]

Autophagy plays an important role in metabolic organs. Insulin signalling activates the mTORC1 pathway not only to stimulate protein synthesis, but also to inhibit autophagy. Although it was hypothesized that insulin deficiency or insulin resistance would increase autophagic activity, insulin resistance is often accompanied by hyperglycaemia, hyperinsulinaemia, dyslipidaemia and other signalling changes that can affect the autophagic activity. The complications of obesity are associated with deregulated mTORC1 activation.[79] Autophagy is suppressed in the livers of mice with insulin resistance and hyperinsulinaemia.[101] Autophagy is insufficient in hepatocytes of patients with obesity or fatty liver.[102] Autophagy is reduced in the mouse hearts of OVE26, a transgenic model of insulinopenic diabetes, and STZ-induced diabetic mouse hearts.[79,103,104] In contrast, autophagy activity in type 2 diabetic hearts is controversial. A study demonstrated that the activities of mTORC1 and AMPK are increased and decreased respectively, and then cardiac autophagy is inhibited in mice with obesity and metabolic syndrome induced by a high fat diet.[105] Other studies demonstrated that cardiac autophagy is unchanged in mice fed a high fat diet,[79] and that a saturated fatty acid myristate promotes ceramide synthase 5-dependent autophagic flux in cardiomyocytes.[106] It is also controversial whether autophagy is adaptive or maladaptive in type 1 and type 2 diabetic models. The study using STZ-injected mice with Beclin 1 haploinsufficiency or Atg16 ablation suggests that the diminished cardiac autophagy is an adaptive response that limits cardiac injury in mouse models of type 1 diabetes.[79] However, chronic metformin therapy significantly enhances autophagic activity and preserves cardiac functions in diabetic OVE26 mice but not in AMPK dominant negative transgenic diabetic mice, suggesting that upregulation of autophagy via AMPK signalling is cardioprotective.[103] The study using high fat-fed mice with cardiac-specific mTOR haploinsufficiency or rapamycin treatment suggests that the diminished cardiac autophagy is maladaptive in high fat diet-induced type 2 diabetes.[105] However, other mechanisms, but not autophagy activation, induced by mTOR haploinsufficiency or rapamycin treatment may have a beneficial effect.

However, the clearance of damaged mitochondria by autophagy and in turn reduction of ROS is an important quality control mechanism under the stressed condition, such as the advanced stage of metabolic cardiomyopathy. When autophagy is suppressed, damaged mitochondria are accumulated and oxidative stress is increased.[88] We recently reported that mTOR hyper-activation by ablation of tuberous sclerosis complex 2 (TSC2) in the mouse hearts induced cardiac dysfunction with the increased number of small mitochondria mediated through the downregulation of autophagy. The upregulation of autophagic flux by trehalose treatment attenuated cardiac dysfunction and structural abnormalities of mitochondria in the TSC2-deficient mouse hearts.[107] The finding suggests that upregulation of autophagy plays an important role in maintenance of cardiac function and mitochondrial homeostasis. Mitochondrial fission, which is a process that divides large mitochondria into smaller daughter mitochondria, segregates dysfunctional mitochondria and creates smaller mitochondria. Mitochondrial fusion serves to mix and unify the mitochondrial compartment for the maintenance of mitochondrial functions.[108] Mitophagy is closely associated with mitochondrial fission.[109] Since the reduced cardiac autophagy in type1 diabetes is associated with increased Rab9 expression in the mitochondrial fraction, it is possible that when canonical autophagy is inhibited, alternative autophagy via Rab9 is upregulated, which may trigger mitophagy to protect the diabetic heart.[79] MtDNA contains inflammatogenic unmethylated CpG motifs.[110] We recently reported that mtDNA that escapes from autophagy caused inflammation and heart failure, and that the TLR9 signalling pathway played a crucial role in recognizing undegraded mtDNA and modulating sterile inflammation in stressed cardiomyocytes.[18] IL-1β and IL-6 are generated when damaged mitochondria are increased and mtDNA degrades insufficiently in autolysosomes in the stressed cardiomyocytes (*Figure 3*).[18] Mitochondria in the failing myocardium also exhibit increased levels of oxidized mtDNA, reduced mtDNA replication and depletion of mtDNA.[111] Oxidized mtDNA into the cytosol during mitochondrial dysfunction may be involved in NLRP3 inflammasome activation (*Figure 3*).[68,69] Thus, since suppression of autophagy leads to cardiac inflammation via dysfunctional mitochondria, upregulation of autophagy may be a therapeutic target in the advanced stage of metabolic cardiomyopathy.

## 5. Closing remarks

In this manuscript, we have discussed the involvement of inflammation in metabolic cardiomyopathy. However, there are still unanswered questions, such as (1) how cardiac inflammation is regulated, (2) whether ablation or reduction of inflammation is a therapeutic target to treat cardiac dysfunction, and (3) whether autophagy is adaptive or maladaptive, in the metabolic cardiomyopathy. *Figure 4* shows current therapeutic strategies of metabolic cardiomyopathy-associated inflammation. Lipid-lowering drugs, such as statins, renin angiotensin aldosterone

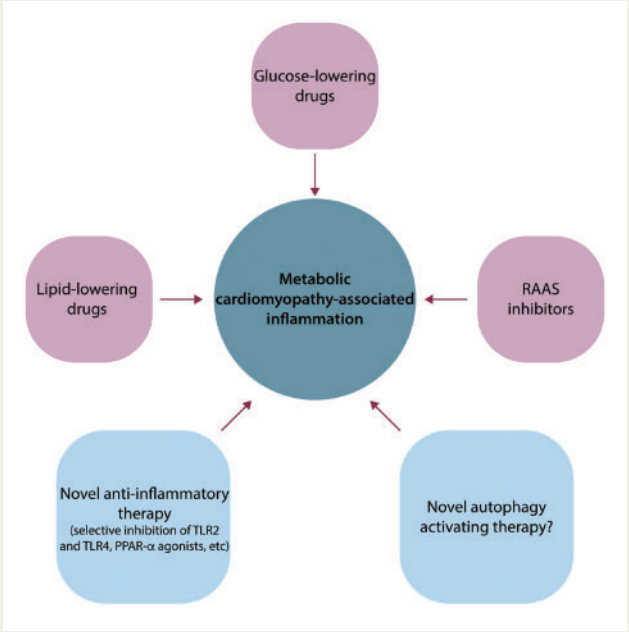


**Figure 4** Therapeutic strategies of metabolic cardiomyopathy-associated inflammation.

system inhibitors, such as angiotensin type 1 receptor antagonists and angiotensin-converting enzyme inhibitors, and glucose-lowering drugs have anti-inflammatory effects.[3,112] It is suggested that the activation of TLR2, TLR4, and NLRP3 inflammasome play an important role in cardiac inflammation and the pathogenesis of metabolic cardiomyopathy. The anti-inflammatory strategies, such as selective inhibition of TLR2 by immunoglobulin G and TLR4 antagonists, attenuate inflammatory response in the models of myocardial contractile dysfunction, such as ischaemia/reperfusion and myocardial infarction,[3] suggesting that they may be novel therapeutic targets for metabolic cardiomyopathy. PPAR-α plays an important role in regulating lipid energy metabolism and its homeostasis in the heart and has anti-inflammatory and antioxidative effects.[113] A PPAR-α agonist, Fenofibrate, increases cardiac autophagy and prevents fibrosis and inflammation in STZ-injected mouse hearts.[114] Another PPARα agonist, Wy14643, prevents the apoptosis induced by glucose and fatty acid in neonatal cardiomyocytes.[42] Resveratrol, a polyphenol produced by plants, has anti-diabetic and antioxidant effects. Resveratrol improves the cardiac function in STZ-induced diabetic rats and mice, and ameliorates the diastolic dysfunction in type 2 diabetic mice mediated through inhibition of TNFα-induced NF-κB activation.[115] These findings suggest that PPAR-α agonists and resveratrol may be effective in the treatment of diabetic cardiomyopathy. Metformin reverses autophagy in the diabetic hearts and autophagy can degrade damaged mitochondria and then prevent inflammation, suggesting that upregulation of autophagy may be a therapeutic target. A better understanding of the regulation of inflammation will potentially generate a novel therapy for preventing or slowing the development and progression of metabolic cardiomyopathy.

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